ABSTRACT

Thermally oxidized oil such as recycled cooking oil and repeatedly used oil were reported to impose deleterious effect to health. In light of the presence of those oils in the market and food preparation process, this study was carried out to differentiate between fresh and thermally oxidized oil and propose parameter that can replace total polar compounds (TPC), the international standard in determining oil degradation status but it is time consuming. In this study, samples were fresh oil, oil subjected to controlled heating and frying in the laboratory at 180 °C to 200 °C for 6 hr and waste oils collected from various food outlets. The differences between fresh and thermally oxidized oil were evaluated based on several parameters; total polar compounds (TPC), fatty acids composition, short chain fatty acids, trans fatty acid, iodine value (IV), free fatty acids (FFA) content, adsorption at 233 and 269 nm under ultra violet (UV) spectrum and oil color. Results showed that fresh and thermally oxidized samples had significantly different level of total polar compound. Color index or absorption at 420 nm showed good correlation (r= 0.848) to TPC but depended on frying parameter especially the food medium. Thermally oxidized oil had decrease in unsaturated fatty acids and increase in saturated fatty acids content. No trans fatty acid was detected in all samples. Short chain fatty acid, the octanoic acid (C8:0) only present in thermally oxidized oil, with correlation of r= 0.750 to TPC. Free fatty acids level showed good correlation (r= 0.863) to TPC but depended on frying parameter especially the moisture content. Iodine value showed acceptable correlation (r = 0.5602) to TPC, however no significant difference between fresh and thermally treated oil. Absorption at 233 and 269 nm, showed correlation of r= 0.8469 and r= 0.8295 to TPC respectively. The presence of octanoic acid (C8:0) was proposed to be used as marker component to differentiate between fresh and thermally oxidized oil as it only present in the later, with simple analytical procedure to be applied as routine analysis and showed good correlation with total polar compounds (r= 0.750).

Keywords: Thermally oxidized oil; Total polar component; Coefficient correlation (r); Fatty acids composition; octanoic acid; Trans fatty acid; Iodine value; Free fatty acids; Adsorption under UV spectrum.

ABSTRAK

Minyak teroksida haba seperti minyak masak kitar semula dan minyak yang digunakan berulang kali dilaporkan memberi kesan buruk kepada kesihatan. Dengan kehadiran minyak tersebut di pasaran dan proses penghasilan makanan, kajian ini telah dijalankan untuk membezakan minyak masak yang belum digunakan dan minyak masak teroksida haba, dan juga untuk mencadangkan parameter yang boleh menggantikan amaun komponan polar, standard antarabangsa dalam menentukan degradasi kualiti minyak masak yang mana kaedah ini memerlukan banyak masa. Di dalam kajian ini, sampel adalah minyak yang belum digunakan, minyak yang dipanaskan dan digoreng di dalam makmal, dengan suhu terkawal antara 180 °C ke 200 °C selama 6 jam dan juga minyak masak terbuang yang dipungut dari beberapa tempat penghasilan makanan. Perbezaan antara minyak masak yang belum digunakan dan minyak masak teroksida haba dinilai berdasarkan beberapa parameter; amoun komponan polar (TPC), komposisi asid lemak, asid lemak rantai pendek, asid lemak trans, nilai iodine (IV), asid lemak bebas (FFA), penyerapan di bawah spectrum UV dan warna minyak. Minyak masak yang belum digunakan dan minyak masak teroksida haba menunjukkan perbezaan amoun komponen polar yang signifikan. Indeks warna atau penyerapan di 420 nm menunjukkan korelasi r= 0.848 kepada amaun komponan polar (TPC) tetapi dipengaruhi parameter sewaktu menggoreng terutamanya medium makanan. Minyak masak teroksida haba menunjukkan penurunan dalam jumlah asid lemak tak tepu dan peningkatan dalam jumlah asid lemak tepu. Tiada asid lemak trans dikesan dalam kesemua sample. Asid lemak berantai pendek iaitu asid ocranoik (C8:0) hanya hadir di dalm minyak masak teroksida haba dengan korelasi r= 0.750 kepada amaun komponan polar (TPC). Jumlah asid lemak bebas (FFA) menunjukkan korelasi r= 0.863 kepada amaun komponan polar (TPC), tetapi bergantung kepada parameter sewaktu proses menggoreng. Nilai iodine (IV), menunjukkan korelasi yang boleh diterima (r= 0.5602) kepada TPC tetapi tiada perbezaan signifikan antara minyak masak yang belum digunakan dan minyak masak teroksida haba. Penyerapan di 233 and 269 nm masing- masing korelasi r= 0.8469 dan r= 0.8295 kepada amaun komponan polar (TPC). Kehadiran asid octanoic (C8:0) dicadangkan sebagai penanda untuk membezakan minyak masak yang belum digunakan dan minyak masak teroksida haba kerana ia hanya hadir di dalam minyak yang terdegradasi, prosedurnya mudah, sesuai untuk diaplikasi dalam analisis rutin dan juga menunjukkan korelasi yang baik dengan amoun komponan polar

Kata kunci: Minyak teroksida oleh haba; Amoun komponan polar; Pekali korelasi (r); Komposisi asid lemak; Asid octanoic; Asid lemak trans; Nilai iodine; Asid lemak bebas; Penyerapan di bawah spectrum UV.

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LIST OF SYMBOLS

°C	degree Celsius
g	gram
kg	kilo gram
m	meter
nm	nano meter
mm	millimeter
µm	micro meter
µL	micro liter
ml	milliliter
%	percentage
hr	hour
min	minute
min^{-1}	per minutes
L	liter
ω	omega
mg	mili gram
cm	centimeter
v/v	volume/ volume
<	less than
r^2	coefficient of determination
r	correlation coefficient
σ	standard deviation
S	slope of calibration curve

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AOCS	American Oil Chemists' Society
CVD	Cardio vascular disease
FA	Fatty acid
FAME	Fatty acid methyl ester
FFA	Free fatty acid
FID	Flame Ionization Detector
FTIR	Fourier transform infra red
GC	Gas chromatography
HDL	High density lipoprotein
IV	Iodine value
IUPAC	International union of pure and applied chemistry
LDL	Low density lipoprotein
LOD	Limit of detection
MSD	Mass spectrometry detector
MUFA	Mono unsaturated fatty acid
PAH	Poly aromatic hydrocarbon
PORIM	Palm Oil Research Institute of Malaysia
PUFA	Poly unsaturated fatty acid

RSD	Residual standard deviation
SFA	Saturated fatty acids
SPSS	Statistical Package for Social Sciences
TAG	Triacylglycerol
TFA	Trans fatty acid
TPC	Total polar compounds
UV	Ultra violet
UV Vis	Ultra violet visible

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Most Malaysians are exposed to unhealthy and unethical food servings whether dining out or at home. For instance, a food manufacturing factory was ordered to closedown for using recycled cooking oil in preparing its processed food products (*The Sun*. 2008. 30 January; *The Star*. 2008. 13 February). Recycled oil, or repeatedly heated and thermally oxidized oil have been well reported to cause deleterious effect to health such as genotoxic, mutagenic, increase of blood pressure, deleterious effect in the endothelial function and carcinogenic (Hageman et al., 1988; Williams et al., 1999; Rueda Clausen et al., 2007; Yildiz et al., 2007 and Leong et al., 2008).

That is the case where the misconduct of food manufacturer was reported. Probably there are many other cases of food manufacturing process that are unreported or proven to be unethical. The recycled cooking oil, besides used as food ingredient for lower cost purpose, might be repackaged and sold as brand new oil in the market. Though have not been proven yet, fast food outlets operators, banana fritters sellers, are notorious for selling their used or discarded oil to third party. It is alleged that the used oils are repackaged to be resold as brand new oil. Consumers who bought this allegedly recycled oil are exposed to anti nutritional properties and deleterious effect of the oil towards health.

A publication written by Riera et al. (2000) claimed, even if recycled oils are used as animal feed, the deleterious effect of the recycled oil can still enter human diet. This is because hazardous compounds in recycled oil such as dioxin, poly aromatic hydrocarbon (PAH) can accumulate over time and through food chain, thus still posing danger to human. This situation shows how consumption of recycled oil can pose deleterious effect to human.

A survey conducted by Norimah et al. (2008) revealed most urban dwellers depend more on food eateries and fast foods outlet for food consumption. The availability of fast food restaurant, 24 hours-eateries has been accounted for this trend. Foods served at these outlets normally are notorious for its processed, fried, and with high calorie and fat content. What more if the foods are prepared by using recycled cooking oil or repeatedly heated oil. Similar to recycled cooking oil, repeatedly heated, or thermally oxidized oil, have been extensively reported to cause adverse effect to health such as cardiovascular disease. It is a normal practice for food outlets operators to use frying oil repeatedly until the oil is fully degraded for cost cutting measure.

A case-controlled study conducted by Iqbal et al. (2008) involving subjects from 52 countries demonstrated that an unhealthy diet, mostly derived from fried food can increase the risk of cardio vascular disease (CVD). The incidence of CVD will become the leading cause of mortality in Malaysia, where 25% of medically certifiable deaths are due to CVD (Rahim, 2009). By 2010, it is projected to be the leading cause of death in Malaysia and other developing countries (*New Straits Times*. 2008. 4 April). This situation shows how dietary habit of Malaysians, whether under or out of their control, especially in terms of fried food, are exposing themselves to cardiovascular disease and other deleterious effects mentioned above. To prevent the hazardous impact of recycled, repeatedly heated cooking oil and increase in cardiovascular disease in Malaysia, changing the dietary habit of Malaysian such as avoiding fried or processed food is deemed to be impossible. Thus, one of the measures that can be taken is controlling the quality of the frying oil itself, which is the compulsory ingredient in fried or processed food. Quality and quantity of fat in foods served at food outlet or food manufacturing company should be monitored especially by health regulatory authorities.

Foods served by outlet operators and food manufacturer usually prepared by deep frying technique. The technique is preferred as it can give valuable sensory taste such as good aroma, crispy, crunchy texture, juicy taste, convenient and relatively low cost in large scale frying (Saguy and Dana, 2003). In practice, frying oil is usually replenished and reused several times prior discarded for lower cost purpose.

However, with the presence of air and moisture during the frying process especially at high temperature between 160°C to 180°C, reusing and recycling the oil will degrade the oil quality as several processes occur such hydrolysis, polymerization, (Bhattacharya et al., 2008; Ramadan, 2010), formation of conjugated dienes, decomposition of hydroperoxides (Yoon et al., 2000). The processes lead to the formation of decomposition product which consists of volatile and non volatile component. Most of the volatile compounds are lost during the frying process while non volatile are of concern as it can accumulate in the degraded oil, absorbed into food, ingested, and enter human diet, causing hazardous effect to health.

Due to the hazardous impact of thermoxidized oil to health, food production process by the food outlet operators and food manufacturer need to be monitored by regulatory authorities to avoid unethical food preparation process such as overused of heated oil, using degraded oil as food ingredient and recycling or selling degraded oxidized oil as a new product.

But the biggest question remain is what is the best, simplest, conclusive method that can be applied as routine analytical approach to determine quality of frying oil. Determination of total polar compounds is recognized as the most reliable method to measure oil degradation (Fritsch, 1981; Marquez Ruiz et al., 1995; Gil et al., 2004; Bansal et al., 2010) and this method has been a standard method to determine oil degradation in some countries (Akoh and Min, 2002).

However, determination of total polar components is time consuming for routine analytical purpose, and the use of large volume of solvents is considered potential environmental problem (Innawong et al. 2004; Ramadan, 2010). These disadvantages are the reason to search for a new generation of rapid methods for the analysis of deep-

frying oils. Other method that correlate well with total polar materials need to be determined to replace this time consuming method.

Rapid test kits to determine oil quality are commercially available. This should solve the problems of time consuming total polar compounds method. However, according to Bansal et al. (2010b), most of the test kits need to be calibrated, so it is really 'rapid' in a real sense. It also based on colorimetric reactions, which is subjective. The biggest setback for these kits is disagreement of results with acceptable conventional wet chemical method.

Physical evaluation such as the odor, color, formation of excessive foaming of the oil is normally conducted and they are the most obvious changes that can be observed even for the non-expert (Abdulkarim et al., 2007). The advantage of physical properties is they are relatively fast and easier to be measured. Moreover, physical properties evaluation usually does not involve hazardous chemicals (Bansal et al., 2010a). However this evaluation depends heavily on the perceptions, judgments of the evaluator and is not reliable between different analysts (Billek et al, 1978).

Chemical methods involve measuring fatty acids composition, free fatty acids value, iodine value, and content of conjugated double bounds (Al Harbi and Al Kahtani, 1993; Gil et al., 2004; Naz et al., 2005; Abdulkarim et al., 2007; Bansal et al., 2010a). All of the chemical methods above depend on the frying parameters such as type of food, oil and the temperature during frying operation. Other factors during frying such as frying temperature, emulsifiers, trace metals, food scraps, free fatty acids and alkaline-reacting materials (Bhattacharya et al., 2008) in the frying oil will cause different type of degradation. Oil consists of fatty acids with a variety in chain length and degree of saturation, so different oil will react different way towards degradation factors.

In addition, chemical method can only roughly determine the quality of oil (Hein et al., 1998), inconclusive and involve measuring non specific compounds. A specific method may be ideal for one operation but completely useless in another (Fritsch, 1981).

Hence, in a study dealing with oil oxidation and degradation, it is desirable to have simple method, independent of factors involve during frying process and will give conclusive results. Specific component need to be detected, regardless of its amount, and will give clear cut difference between unused oil and degraded oil, and can act as marker for oil degradation.

This present study was carried out to understand oil degradation level after thermally treated and oxidized. Several chemical parameters that have been used in literatures in determining oil degradation status will be tested. The assessment parameters were fatty acid composition and saturation level of the oil, presence of trans and short chain fatty acid, free fatty acid content, iodine value, level of conjugated fatty acid. These parameters were tested to determine which one correlates well with total polar compounds and can replace the time consuming method.

Statistical analysis was conducted to determine correlation between those chosen parameters and total polar compounds. The assessment parameters also were evaluated to determine which one is the simplest to be applied as routine analytical procedure, and can give conclusive, clear cut different between fresh and used oil regardless of oil type and frying parameters.

Palm oil was chosen as sample in this study owing to their common use as cooking medium by Malaysian. The samples in this study were fried oils collected from several food outlets. These fried oils were different in terms frying parameters and condition such as frying temperature, type of food, type of oil and fryer dimensions. This is to signify different frying variables and condition that can influence type and rate of oil degradation.

Oil samples also were subjected to frying and heating in laboratory under controlled condition. These samples acted as control sample in this study. Frying and heating were compared to understand the influence of food towards oil degradation. Corn oil also was included as sample control in this study because it is relatively has

high activity towards oxidation compared to palm oil, and comparison was made between these two oils.

1.2 PROBLEM STATEMENT

Quality of edible oil sold in the market and oil used in food manufacturing process and food outlets are questionable. The quality should be regulated by health regulatory bodies. But the question remains, what is the best method to determine oil degradation status. Several analytical approaches are available to determine oil deterioration level. However those methods are time consuming, not suitable for routine analysis, depends on oil type and frying condition, and some could not give conclusive results. Most published researches on degraded oil mostly conducted on corn, canola and soya bean oil which properties are different from palm oil, the main cooking oil consumed by Malaysian. Thus the results from those studies could not be applied on palm oil. Previous analysis normally conducted by severe heating and condition of oil in laboratory, which is not representative of real frying condition, and no sampling at food outlets were conducted.

This study was carried out to understand palm oil degradation level. Oil samples were collected from various food outlets that are usually patronized by Malaysian and notorious for using oil until fully degraded. It is also to determine simple, conclusive method that can be applied as routine analysis to evaluate palm oil degradation status and replace total polar compounds as standard method as this method is reliable but time consuming for routine analysis.

1.3 OBJECTIVE

- To determine properties of fresh and thermally oxidized palm oil
- To test available analytical methods in oil degradation evaluation to determine the best and simplest method that can be applied as routine analysis in determining palm oil degradation level.
- To determine component that is conclusive and can act as marker to degraded oil

1.4 HYPOTHESIS

- Fresh and thermally oxidized oil have different properties in terms of fatty acid composition, total polar compounds, iodine value, free fatty acid, color formation.
- Analysis using wet chemical methods such as total polar compounds, iodine value, free fatty acids are expected to be time consuming compared to analysis run by instrument such as gas chromatography and UV spectrophotometer. Color formation, free fatty acid, iodine value, total polar compounds, conjugated fatty acid, changes in fatty acid composition is expected to be influenced by oil and food composition
- Octanoic acid, a short chain fatty acid, can act as marker to differentiate between the fresh and degraded oil.

CHAPTER 2

LITERATURE REVIEW

2.1 FATS AND OIL

Fats and oil are major nutrient in the human diet and more than 90% of global oil production is used as food or as ingredient in food products (Moreno et al., 1999). Oils and fats consist mostly of more than 95% of triacylglycerol (Heldman and Lund, 2007; Othmer, 2008). One molecule of triacylglycerol (TAG) composed of three fatty acids esterified to a glycerol, hence its name. The hydroxyl group of the glycerol joins with carboxyl groups of the fatty acid to form ester bonds (Akoh and Min, 2002). The three fatty acids may or may not be identical, thus an edible fat or oil may contain more than 500 different TAG (Heldman and Lund, 2007). Figure 2.1 illustrates how fatty acids are connected to one molecule of glycerol to form TAG in oil.

Gilbert et al. (2009) and Othmer (2008) defined fats as triacylglycerol that composed primarily of saturated fatty acids and solid at room temperature, while oils are composed predominantly of unsaturated fatty acids and liquid at room temperature.In food preparation, oils and fats contribute to flavor and palatability (Akoh and Min, 2002) while during frying, oil acts as heat transfer medium (Al Harbi and Al Kahtani, 1993).

Oxidized oils and fats are responsible for rancidity, development of off flavor, loss of fat soluble vitamin and pigment in foods (Heldman and Lund, 2007), production of toxic compounds and can cause the oil and fats to become unacceptable for consumers for edible or other use (Othmer, 2008).

In biological aspect, fats and oil supply energy, support structural aspects of the body, and provide substances that regulate physiological process. However excess in fats in the body can produce harmful effect (Akoh and Min, 2002).

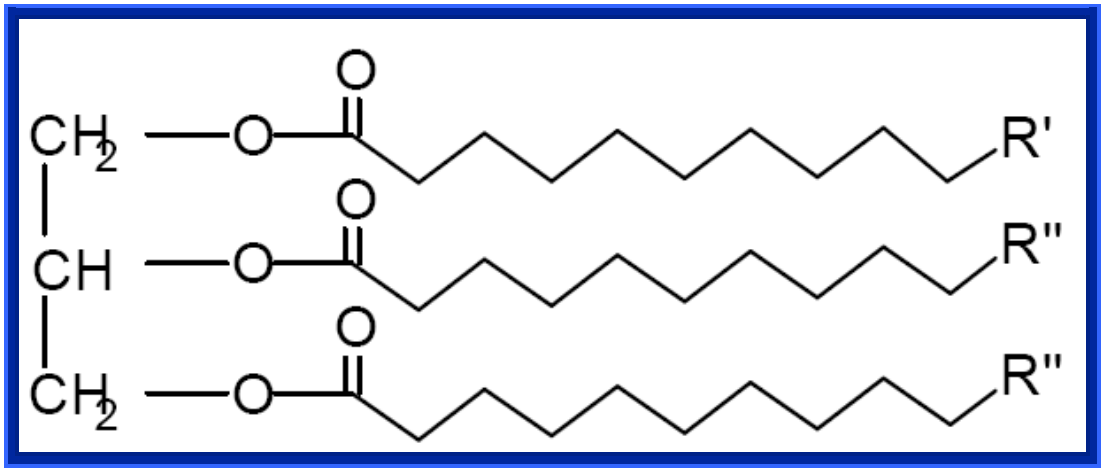

Figure 2.1: Structure of triacylglycerol in oil.
R', R'' and R''' indicate fatty acid chain length.

2.2 PALM OIL

Palm oil is widely used in various food products, such as margarines, shortenings, cooking oils, confectionery fats and vanaspati (Saad et al., 2007). Most Malaysian are consumers of palm oil. Corley (2009) stated that Malaysia has highest palm oil per capita consumption at 183 kg/head, compared to other region such as China with 10.6 kg, 17.7 kg in India and 39.3 kg in the USA. This high consumption might be due to its relatively low in price compared to other vegetable oil.

Survey in hypermarket chain also confirmed that the price is cheaper compared to other vegetable oil. This is in line with status of Malaysia as a leading producer of palm oil in the world (Basiron, 2002; Othmer, 2008; Abdullah et al. 2009).

Palm oil is semi solid at room temperature, and palm olein, which is fraction of palm oil, is widely used for industrial frying (Berger and Idris, 2005; Bansal et al.,

2010a). It is commonly used for preparation of fried foods due to its availability and better stability (Bhattacharya et al., 2008).

Palm oil occupies an intermediate position among natural fats as its saturated acid is 50% and the rest is unsaturated fatty acid. The fatty acid composition contributes to palm oil semi solid properties (Yadav, 2006), thus it is not required to undergo hydrogenation process, which is process of oil hardening, and can result in formation of trans fatty acids.

Trans fatty acid is the main issue in edible oil industry, as it is said to be deleterious to health. The move away from trans-fatty acids favors palm oil, as it can be used without hydrogenation (Muller et al., 1998; Tang, 2002; Berger and Idris, 2005; Moraes Mizurini et al., 2010). Palm oil contains high level of saturated fatty acids and it is the most important edible source of palmitic acid or hexadecanoic acid (C16:0), which is saturated fatty acid (Bautista et al., 2001). Its saturated and unsaturated fatty acid ratio is close to one and contains a considerable amount of carotene, an antioxidant (Noh et al., 2002).

Palm oil usually is consumed in the oxidized state due to heat exposure during cooking (Leong et al., 2008). In terms of susceptibility towards oxidation, palm olein and palm oil are low in sensitivity compared to oil with high unsaturated fatty acids contents (Al Harbi and Al Kahtani, 1993). Therefore it is widely used as a long-lived frying medium and gaining popularity in food industry (Berger and Idris, 2005; Leong et al. 2008).

Leong et al. (2008) observed that prolonged consumption of repeatedly heated palm oil may result in an increase in blood pressure level with necrosis of cardiac tissue. Al Harbi and Al Kahtani (1993) studied effect of feeding discarded palm oil sample to rats. They discovered oil palm did not give effect as deleterious as other oil with high polyunsaturated fatty acids contents, this is in contrast to research carried out by Rueda Clausen group in 2007. They discovered palm oil gave similar effect with oil with high unsaturated contents like sun flower and olive oil.

2.3 DEEP FRYING METABOLISM AND ITS EFFECT

Deep frying involves the process of immersing food item in large quantity of heated oil (Al Harbi and Al Kahtani, 1993). It is one of the most popular procedure for food preparation since it is rapid, develops desirable flavor, color and texture (Takeoka et al. 1997; Rossi et al. 2009). In cooking process, frying is favored over other methods. This is because, in roasting or oven cooking, the heat conductor is the air, but air is not a good heat conductor, so cooking with this method is a slow process (Berger, 2005) while cooking with oil will result in more rapid penetration of heat into the product being cook (Stevenson et al., 1984). For boiling process, the temperature of cooking is limited to the boiling point of water which is 100 °C, thus desirable crispy texture would not be achieved. Meanwhile, immersing food in hot oil can retain all the food flavors and juices within the crispy crust (Sanchez-Gimeno et al., 2008), thus frying can produce crispy texture at the outside and juicy texture at the inside.

Frying operation normally is conducted at 175–195 °C (Saguy and Dana, 2003; Alvis et al., 2009). Heat and mass transfer occur during frying and the amount depends on factors such as temperature, warm up time, oil type, food, oil rotation, its manipulation, and finally, the equipment used (Alvis et al. 2009; Rossi et al. 2009). Heat is transferred from the hot oil to the surface of the food material, while moisture is transferred from the food interior to the food surface (Yildiz et al., 2007). It is also considered food dehydration process and more exactly a procedure of water extraction by convection with change of state (Alvis et al., 2009).

With repeated frying, the presence of moisture, heat, the incorporation of oxygen into triacylglycerol structure in the oil can cause occurrence of several processes. If oxygen is scarce, thermolytic reactions will take place. In the presence of air, both oxidative and nonoxidative reactions will occur simultaneously, while heat will accelerate the changes (Nawar, 1984).

During frying, water presence in food will vaporize and the steam that is formed will hydrolyze triacylglycerol, forming free fatty acids (Bhattacharya et al., 2008).